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by

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16. ABSTRACT
A purified preparation of the simian rotavirus SA-11 containing mostly single virion particles and a preparation of cell associated SA-11 virions were tested for their resistance to inactivation by three disinfectants. With chlorine, both virus preparations were inactivated more rapidly at pH 6 than 10. With chlorine dioxide both virus preparations were inactivated more rapidly at pH 10 than 6. Monochloramine at pH 8 inactivated both virus preparations but at a much slower rate than chlorine or chlorine dioxide.

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EFFECT OF CHLORINE, CHLORINE DIOXIDE AND MONOCHLORAMINE ON ROTAVIRUS (SA-11) INACTIVATION

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Rotaviruses were first visualized by electron microscopy in 1973 (1). Because the virus had the appearance of a wheel with radiating spokes it was named rotavirus, however, it has also been called: orbivirus, duovirus, reovirus - like agent and infantile gastroenteritis virus (2). The virus is now recognized as a causative agent of infantile gastroenteritis. The virus is ubiquitous and rotavirus agents have been isolated from animals such as mice, monkeys, calves, pigs, lambs, rabbits, deer and antelope (3). Some of the reported properties of rotavirus suggest the possibility that this virus could be waterborne. These properties are: (a) the virus is shed in feces in high titer, up to 10^{10} virus particles per gram and it is apparently shed in large clumps (4, 5); (b) the virus is environmentally stable in natural waters (6); (c) it does not adsorb as efficiently as poliovirus 1 to aluminum hydroxide and activated sludge flocs (7); (d) the virus is resistant to acid, ether, chloroform, freezing, thawing, genetron and is stable below pH 10 (8); (e) the virus is heat stable at 60°C for 1 hour (9); (f) it is apparently resistant to disinfectants (10, 11). Brade et al. (12) found that only 2 of the 5 commercial disinfectants that they tested were effective for inactivation of the rotavirus SA-11.

This study was initiated because of reports that rotaviruses are resistant to common disinfectants. It is important to know if chlorine or suggested alternative disinfectants for water treatment such as chlorine dioxide and monochloramine inactivate rotaviruses. In this study the simian rotavirus SA-11 was used as a model for the human rotavirus because the human rotavirus does not grow well in cell cultures.

MATERIALS AND METHODS

Preparation and Purification of Stock Virus

The simian rotavirus SA-11 was obtained from Dale Van Donsel of the U.S. Food and Drug Administration, Cincinnati, Ohio. The virus was grown and assayed in our laboratory in BGM cell cultures. For preparation of stock virus, BGM cells were grown in 690 cm^2 roller bottles then washed 3X with 50 mL portions of a medium containing 50% Eagle's minimum essential medium (MEM) and 50% Leibovitz medium (L-15). This combined medium was designated MEM-L-15. The cultures were drained of media, and then inocula ted with

rotavirus at an approximate multiplicity of infection of 0.4 virus/cell. The inoculum was spread evenly over the surface of the cell monolayer by rotating the bottles by hand. An additional 50 mL of medium was added and the bottles were placed on a roller apparatus and incubated at 37°C. When 75-100% of the cells showed viral-induced cytopathogenic effects (CPE) the cultures were frozen and thawed 3X, then sonicated at 100 watts for 1 minute. Cellular debris was removed by centrifugation for 30 minutes at 1300 x g. For purification purposes, equal volumes of the viral supernatant and freon 113 were mixed in a Virtis blender at a setting of 60 for 1 minute. After centrifugation for 10 minutes at 1100 x g, the supernatant (upper aqueous layer) was removed by aspiration and sufficient 0.06M $MgCl_2$ was added to it to reach a final concentration at 0.006M. The pH was next adjusted to 3.0-3.1 with 1 N HCL and the preparation was filtered through a 47 mm, 0.45 um HA Millipore membrane that had been previously washed with sterile distilled water. The membrane containing adsorbed virus was washed with 0.006M $MgCl_2$ at pH 3.0 to remove residual chlorine demand substances. The virus was eluted from the membrane with demand free water that had been adjusted to pH 11.4. The eluted virus was collected in 5 mL of 0.05M KH_2PO_4 buffer at pH 7. The purified virus had a negligible chlorine demand and was frozen in 2 mL portions at -70C.

Cell associated virus was prepared in 75 cm^2 flasks of BGM cells. The cultures were washed 3X with MEM-L-15 media then drained of media and inoculated with 8 mL of stock virus/flask. The multiplicity of infection was approximately 20. After 15 minutes incubation at room temperature, 20 mL of medium was added and the cultures incubated at 37°C. When 50-100% of the cells showed CPE, the cultures were refrigerated for 30 minutes and the cells were scraped from the flasks using a rubber policeman. The cellular material was centrifuged at 1100 x g for 10 minutes and the supernatant discarded. The sediment was resuspended in 50 mL of chlorine demand-free 0.05M KH_2PO_4 buffer, pH 7 and recentrifuged. The supernatant was again discarded. After a second wash with buffer, the cell associated virus was resuspended in 25 mL of phosphate buffer and refrigerated at 4°C until used in experiments. The chlorine demand of this virus preparation was neglible.

Assay for Virions

Virions were assayed by the plaque technique (13) in BGM cells grown in 25 cm^2 flasks. The cultures were washed 3X with MEM-L-15 media then drained of media and inoculated with 1 mL of virus sample. Viral dilutions were made in the MEM-L-15 media. After 1 hour of incubation at room temperature, the inoculated cultures were overlaid with a medium consisting of Eagle's MEM containing 1.5% (w/v) purified agar (Oxoid) 15 ug/mL neutral red, 0.15% (w/v) $NaHCO_3$, 100 ug/mL DEAE dextran (MW 2 x 10^6) and 0.001M $CaCl_2$. Pancreatin was also added to the overlay medium. To accomplish this, one buffered pancreatin tablet (Oxoid) was dissolved in 50 mL of water and filtered through a 0.45 um pore size membrane. This pancreatin stock solution was added to the overlay media at a rate of 1% (w/v).

Stock chlorine solutions (approximately 1 mg/mL) were prepared either from Clorox or laboratory grade sodium hypochlorite

solution. The chlorine concentration was determined by the DPD method (14). The stock chlorine dioxide solution was prepared from the gas generated by adding dilute H_2SO_4 to a sodium chlorite solution (14). The chlorine dioxide concentration was determined by the DPD Method (14). Stock monochloramine solutions were prepared weekly by combining a chlorine solution and a solution of $(NH_4)_2SO_4$. The mixture ratio was 4 parts of ammonia to 1 part of chlorine. Monochloramine concentration was also determined by the DPD method (14). For the disinfection experiments, the stock monochloramine solution was diluted in the phosphate buffer at pH 8 to a concentration of about 10 mg/liter.

Disinfection Test Procedure

The pH of the phosphate buffer (0.05 M KH_2PO_4) used for the pH 6 and 8 studies was adjusted to the desired pH level with 10 M NaOH and then made chlorine demand free by adding sodium hypochlorite to it until a free chlorine concentration of 1-2 mg/liter was obtained. The buffer was kept at room temperature 1 or 2 days then boiled for 5 minutes. After cooling it was dechlorinated by exposure to UV radiation (254 nm) for 24-48 hours. When no free chlorine was detected in a spot test with orthotolidine, the buffer was considered demand free. Boric acid buffer (0.05M), used only for experiments at pH 10 was prepared from boric acid, and was made demand-free in the same manner as the phosphate buffer with the exception that the pH was adjusted after the solution had been made demand-free. For test purposes, quantities of the appropriate stock disinfectant were added to the demand-free buffer. Disinfectant supplemented and unsupplemented buffers were placed in beakers and with the remaining buffer were refrigerated overnight. The disinfectant concentration was determined the following day and was readjusted when necessary. A multiple stirring device equipped with stainless steel paddles operating at full power was used for stirring samples during the disinfection experiments. The refrigerated buffer in the beakers was used to rinse the stirring paddles. The buffer in each beaker was then discarded. The beakers were refilled with the remaining buffer and placed in a 5°C water bath. After the temperature had equilibrated, stirring was started and 1 mL of the desired test virus suspension was added. At timed intervals, samples were pipetted into the 0.05M, pH 7 phosphate buffer which contained 1.25 mg of sodium thiosulfate/mL as a neutralizer for the disinfectant. The buffer was then assayed for virus survival. The residual disinfectant concentration was also determined at timed intervals. Control samples consisted of buffer supplemented with disinfectant that had been neutralized and then inoculated with virus, and unsupplemented buffer inoculated with virus. The virus titers obtained from the two controls for each test were averaged and the titer obtained was considered as 100% survival. Percent survival at each timed interval was calculated and the results (% survival, vs. time) were plotted on semilog paper.

Two cell culture controls were employed for each experiment. For one control, the cultures were inoculated with a stock suspension of SA-11 (about 30 PFU/mL). This control verified the susceptibility and sensitivity of the cell cultures to the virus. The purpose of the second control was to demonstrate that the cell cultures contained no indigenous viruses or other agents

which might appear to cause viral plaques. To accomplish this, uninoculated cultures were overlaid and examined daily for plaques or other agents. A chlorine quality assurance check sample supplied by the Environmental Monitoring and Support Laboratory of U.S. E.P.A., Cincinnati was also tested on a routine basis. The assay of this sample demonstrated that our spectrophotometer and reagents were giving accurate assay values.

RESULTS

Figure 1 shows an electron micrograph of purified SA-11 containing mostly single virion particles. This is important because the shape of the disinfection curve is dependent on the state of aggregation of the virus. Repeated tests showed that the purified virus was very sensitive to chlorine at pH 6 and 5°C. More than 4 logs (99.99%) of the virus was inactivated in less than 15 seconds by 0.5, 0.25 and 0.1 mg/L of chlorine. However, when this virus was tested in borate buffer at pH 10 and 5°C, the time required for 99% inactivation of the virus was about 1.4 minutes (Figure 2).

The time required for 99% inactivation of SA-11 by 1 and 0.5 mg/L ClO_2 at pH 6 and 5°C was 12 and 23 seconds respectively. Thus, chlorine dioxide at pH 6 did not inactivate SA-11 as rapidly as chlorine. At pH 10, however, 0.5 mg/L of chlorine dioxide inactivated more than 99.99% of the virus in less than 15 seconds.

The purified SA-11 virus was apparently resistant to 10 mg/L of monochloramine at pH 8 and 5°C. Figure 3 shows that 99% of the virus was inactivated in about 6.75 hours.

Figure 4 shows an electron micrograph of a cell associated preparation of rotavirus SA-11. The virus along with the cellular debris appears to be in large clumps. Disinfection experiments with the cell associated SA-11 shows that inactivation occurred at a slower rate than with the purified preparation of free virions. A series of 7 experiments with cell associated virus and 0.5 mg/L of chlorine at pH 6 and 5°C gave an average time of 1.75 minutes for 99% inactivation of the virus (range 1.10 - 2.38 minutes). At pH 10, the average time for 99% inactivation of the cell associated virus with 0.5 mg/L chlorine was 3.5 minutes (range 2.5 - 4.4 minutes). The type of disinfection curve obtained under these conditions is shown in Figure 5.

The cell associated SA-11 virus was tested for its resistance to 0.5 mg/L of chlorine dioxide at pH 6 and 5°C. At pH 6, the average time for 99% inactivation of the virus was 2.9 minutes with a range of 1.9 to 4.7 minutes. The time for 99% inactivation at pH 10 was about 0.4 minute (range 0.3 - 0.42 minutes).

The results of a typical experiment with the cell associated SA-11 virus preparation and 10 mg/L of monochloramine at pH 8 and 5°C is shown in Figure 6. The average time for 99% inactivation of the virus was 10.2 hours (range 9.5 - 10.6).

The time required for 99% inactivation of the purified and cell associated SA-11 virus by chlorine at pH 6 and 10 is summar-

ized in Table 1. For comparison purposes, the results of Engelbrecht et al. (15) with poliovirus 1 and coxsackievirus A-9 are also summarized in Table 1.

Discussion

Early literature reports (10, 11) indicated that rotaviruses were resistant to common disinfectants, including chlorine. However, these experiments were not done with purified virus preparations and were not adequately controlled. In contrast, a recent communication (16) stated that the human rotavirus strain Wa is inactivated rapidly by a chlorine type disinfectant. Our results agree with this conclusion and show that purified simian rotavirus SA-11 is very sensitive to free chlorine at pH 6 and 5°C. The inactivation rate was too rapid to measure even at 0.1 mg/L chlorine. Because the pH of the disinfecting solution has an effect on the species of chlorine present and an effect on the virus as well, inactivation experiments were also conducted at pH 10. Even though virus survival time was greater at pH 10, 99% of SA-11 was inactivated by 0.5 mg/L of chlorine in less than 2 minutes (Figure 2).

Chlorine dioxide is also an effective disinfectant against the purified SA-11 virus. More than 99% of the virus was inactivated by 0.5 mg/L ClO_2 at pH 6 in less than 1 minute. At pH 10, the rate of inactivation with 0.5 mg/L of ClO_2 was too rapid to measure. More than 99% of the virus was inactivated in less than 15 seconds. Thus, the virus inactivation rate increased with an increase in pH from 6 to 10. With chlorine, the opposite was true, namely the virus inactivation rate decreased with an increase in pH from 6 to 10.

Hoff (17) reported that cell associated poliovirus was more resistant to chlorine than freely suspended virions. We found this to be true with the SA-11 virus also. However, even with the protection afforded by cellular debris, 99% of the virus was inactivated by 0.5 mg/L of chlorine at pH 6 and 5°C in less than 2 minutes (average time of 1.75 minutes for 7 experiments). At pH 10, 99% of the virus was inactivated in 3.5 minutes (Table 1). Thus, the pH effect was also evident with the cell associated virus preparation. The virus inactivation rate with chlorine decreased with an increase of pH from 6 to 10. The cell associated rotavirus was more resistant to inactivation with chlorine at pH 6 and 10 than the purified SA-11 virus preparation.

Chlorine dioxide inactivated 99% of the cell associated virus preparation in about 3 minutes at pH 6. The time for 99% inactivation decreased to 0.4 minute at pH 10. The virus inactivation rate with ClO_2, therefore, increased with an increase of pH from 6 to 10. The cell associated rotavirus was more resistant to inactivation with chlorine dioxide at pH 6 and 10 than the purified SA-11 virus preparation.

The cell associated rotavirus was also more resistant to monochloramine than the purified SA-11 virus preparation. The time for 99% inactivation of the cell associated virus was about 10.2 hours with 10 mg/L of monochloramine compared to 6.75 hours for the purified virus. Two additional controls were added during these tests with monochloramine. One control consisted of the

test monochloramine solution inoculated with the purified SA-11 preparation. This biological control would indicate the presence of free chlorine in the test monochloramine solution because that virus preparation is very sensitive to free chlorine. There was virus survival for an hour and longer, indicating that no free chlorine was present. The second control contained the $(NH_4)_2SO_4$ solution added to distilled water (instead of chlorine) and then diluted for test purposes as if it contained monochloramine. This solution did not inactivate the SA-11 virus, indicating that the inactivation obtained with monochloramine was due to the monochloramine and not to unreacted $(NH_4)_2SO_4$.

The results of these studies compare favorably with the results reported by Engelbrecht (15) on the inactivation of several enteroviruses with chlorine.

The rapid inactivation of the simian rotavirus SA-11 by chlorine indicates that this rotavirus should pose no problem in the disinfection process used in water supply operations. Alternative disinfectants such as chlorine dioxide and monochloramine are also effective disinfectants for this virus provided adequate contact time is established. It should be remembered, however, that the SA-11 virus was used only as a model for the human rotavirus. The resistance of the human rotavirus to chlorine and other disinfectants should be studied.

This paper has been reviewed in accordance with the U.S. Environmental Protection Agency's peer and administrative review policies and approved for publication.

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REFERENCES

1. Bishop, R. F., G. P. Davidson, I. H. Holmes and B. J. Ruck. 1973. Virus Particles in Epithelial Cells of Duodenal Mucosa from Children with Non-Bacterial Gastroenteritis. Lancet, 11, 1281-1283.

2. WHO Scientific Working Gorup. 1980. Rotavirus and other Viral Diarrhoeas. Bull. World Health Org. 58 (2), 183-198.

3. McNulty, M. S. 1978. Rotavirus. J. Gen. Virol. 40, 1-18.

4. Cutting, W. A. M. 1979. Viral Gastroenteritis in Children. Trop. Doctor. Jan. 16-20.

5. Narang, H. K. and A. A. Codd. 1981. Frequency of Preclumped Virus in Routine Fecal Speciemens from Patients with Acute Non Bacterial Gastroenteritis. J. Clin. Microbiol. 13 (5), 982-988.

6. Hurst, C. J. and C. P. Gerba. 1980. Stability of Simian Rotavirus in Fresh and Estuarine Water. Appl. Environ. Microbiol. 39 (1), 1-5.

7. Farrah, S. R., S. M. Goyal, C. P. Gerba, R. H. Conklin and E. M. Smith. 1978. Comparison Between Adsorption of Polyvirus and Rotavirus by Aluminum Hydroxide and Activated Sludge Flocs. Appl. Environ. Microbiol. 35 (2), 360-363.

8. Estes, M. K., D. Y. Graham, E. E. Smith and C. P. Gerba. 1979. Rotavirus Stability and Inactivation. J. Gen. Virol. 43, 403-409.

9. Van Donsel, D. J. and E. P. Larkin. 1980. Thermal Inactivation Studies on Bovine and Simian Rotavirus. In: Abstracts of the 1980 Annual Meeting of the ASM.

10. Snodgrass, D. R. and J. A. Herring. 1977. The Action of Disinfectants on Lamb Rotavirus. Vet. Rec. 101, July 23, p. 81.

11. Tan, J. A. and R. D. Schnagl. 1981. Inactivation of Rotavirus by Disinfectants. Med. J. Australia 1, June 10, 19-23.

12. Brade, L., W. A. K. Schmidt, and I. Gattert. 1981 Relative Effectiveness of Disinfectants Against Rotaviruses. Zbl. Bakt. Hyg., Abt. Orig. B, 174: 151-159.

13. Dahling, D. R., G. Berg, and D. Berman. 1974. BGM, A Continuous Cell Line More Sensitive Than Primary Rhesus and African Green Kidney Cells for The Recovery of Viruses from Water. Health Lab Sci. 11 (4), 275-282.

14. American Public Health Association. 1975. Standard Methods for the Examination of Water and Wastewater, 14th Ed. American Public Health Association, Inc., New York.

15. Engelbrecht, R. S., M. J. Weber, B. L. Salter and C. A. Schmidt. 1980. Comparative Inactivation of Viruses by Chlorine. Appl. Environ. Microbiol. 40 (2), 249-256.

16. Tan, J. A. and R. D. Schnagl. 1983. Rotavirus Inactivated by a Hypochlorite-based Disinfectant: A Reappraisal. Med. J. Australia. 1, June 11, 550.

17. Hoff, J. C. 1978. The Relationship of Turbidity to Disinfection of Potable Water. Evaluation of Microbiology Standards for Drinking Water. C. H. Hendricks, ed., EPA-570/9-78-00C.

TABLE 1. Comparison of virus inactivation by 0.5 mg/liter of chlorine at pH 6 and 10 and 5°C.

Virus Type	Time for 99% inactivation (min.)	
	pH 6	pH 10
Poliovirus 1*	2.1	21
Coxsackievirus A-9*	0.3	1.5
Purified SA-11 (single virions)	< 0.25	1.4
Cell associated SA-11	1.75	3.5

*From: Engelbrecht et al. 1980. Appl. Environ. Microbiol. 40 (2), 249-256.

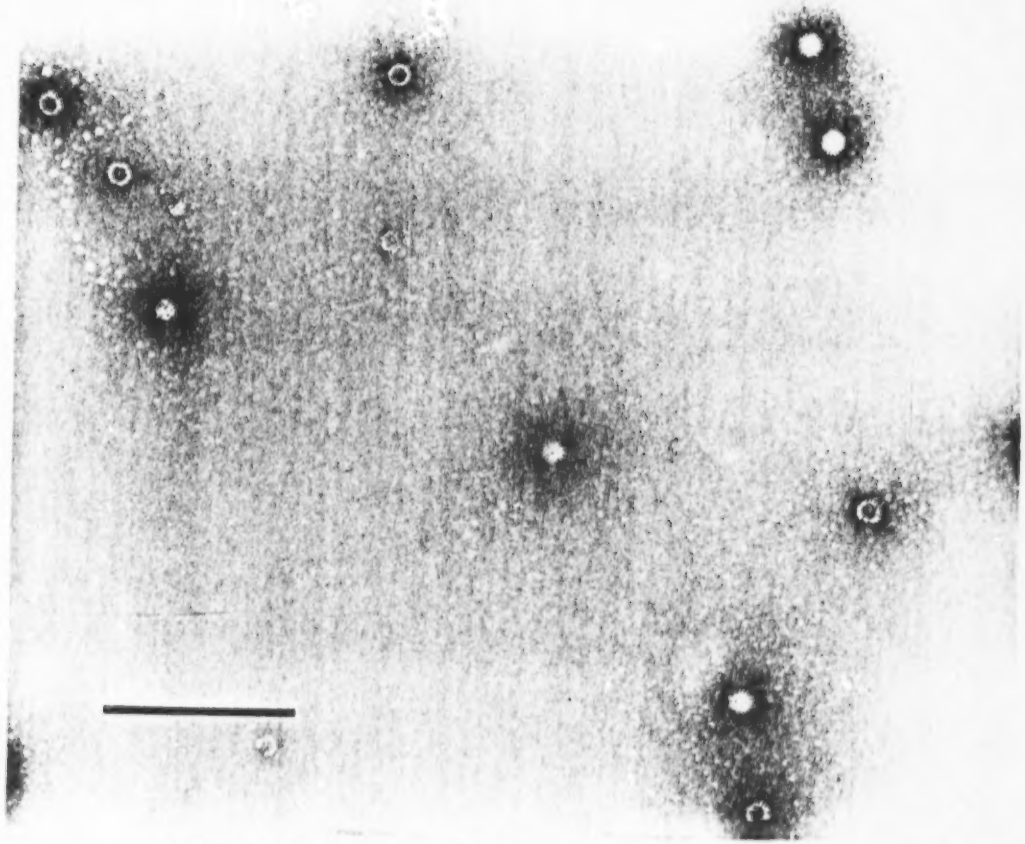

FIGURE 1. ELECTRON MICROGRAPH OF PURIFIED SIMIAN ROTAVIRUS SA-11. BAR = 500 nm

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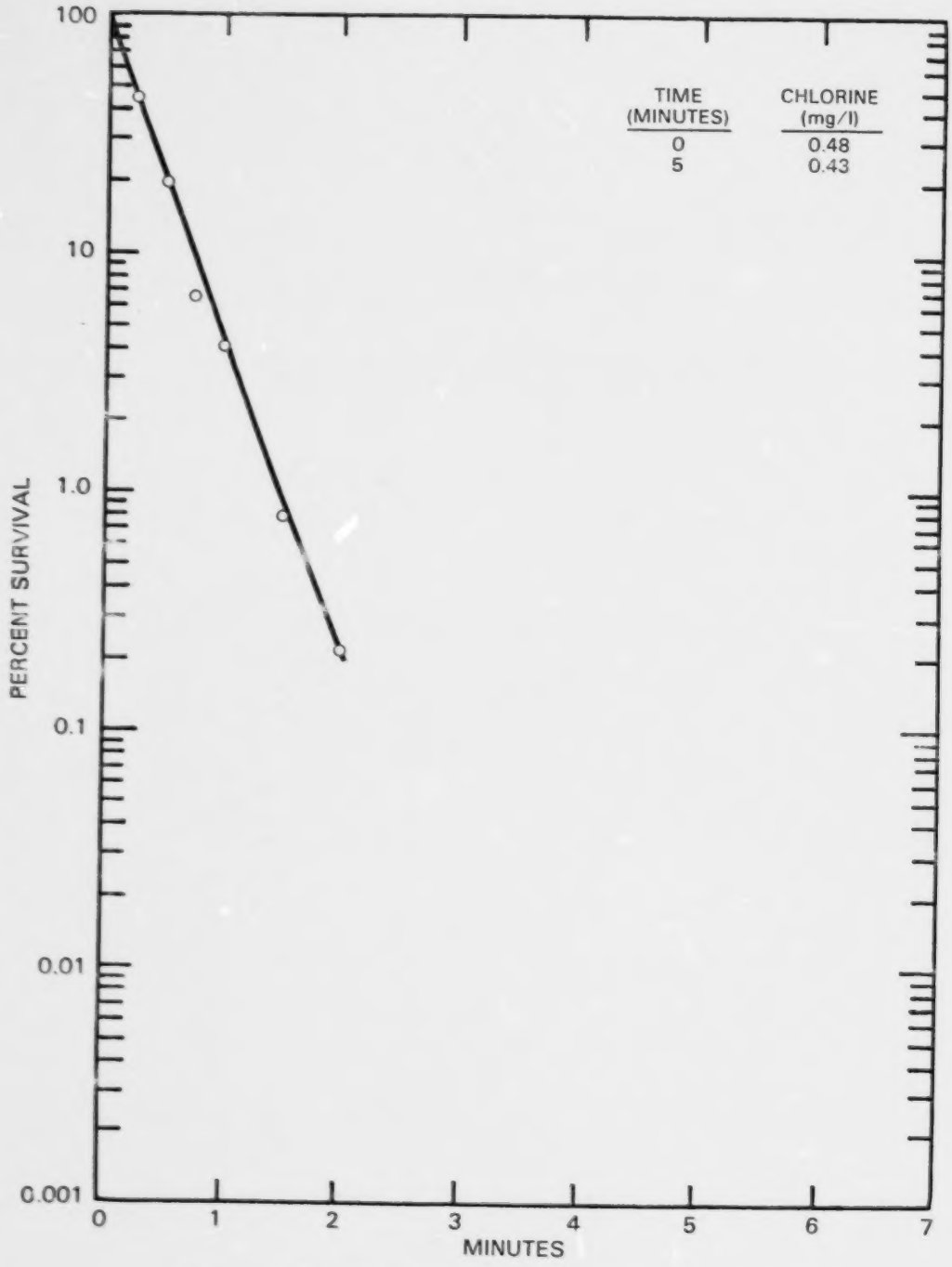

FIGURE 2. THE EFFECT OF 0.43 MG/LITER CHLORINE AT pH 9.9 AND 5°C ON PURIFIED SA-11.

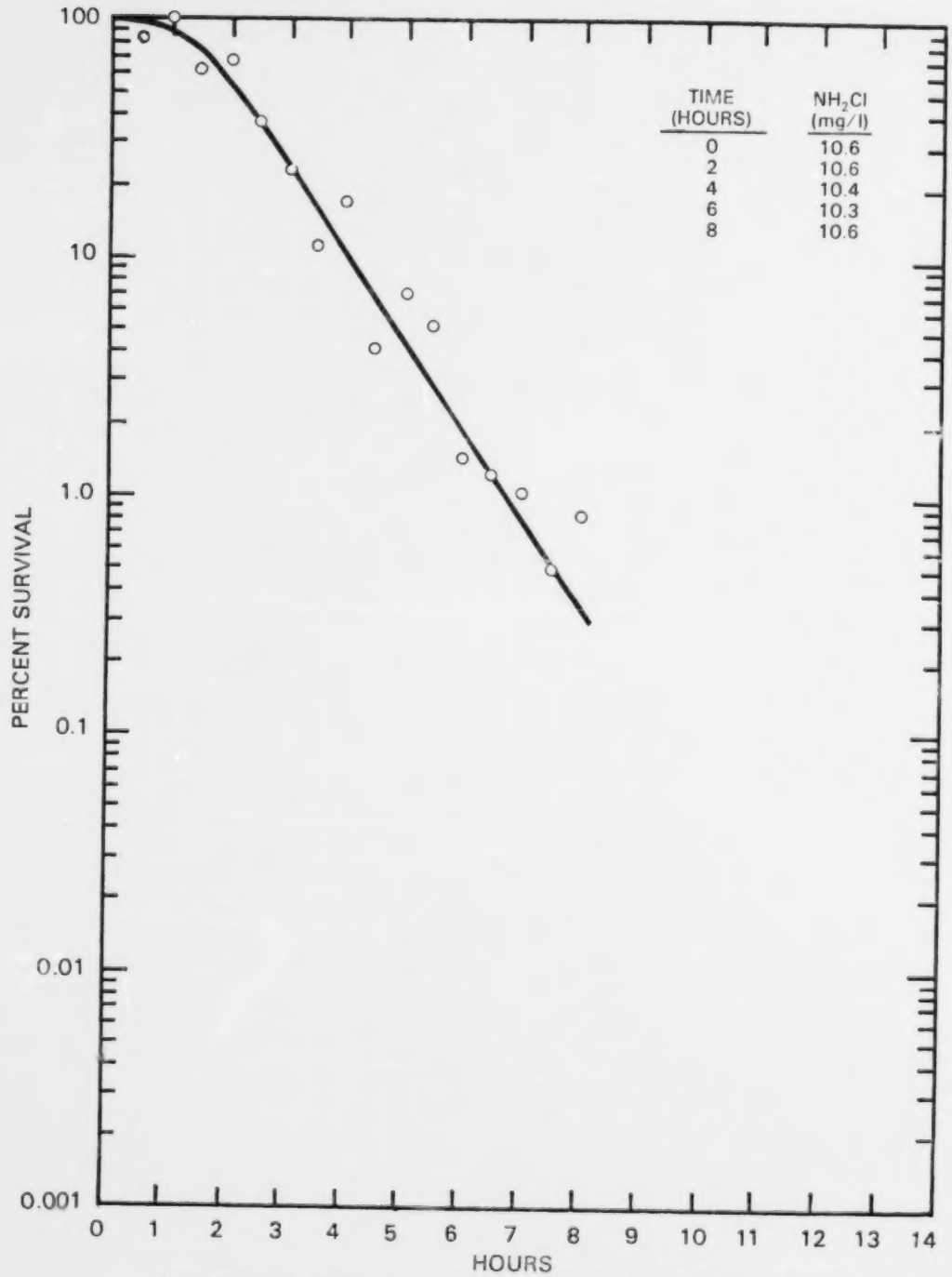

FIGURE 5. THE EFFECT OF 10.6 MG/LITER MONOCHLORAMINE AT pH 8 AND 5°C ON PURIFIED SA-11.

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FIGURE 4. ELECTRON MICROGRAPH OF CELL ASSOCIATED SIMIAN ROTAVIRUS SA-11.
BAR = 500 NM.

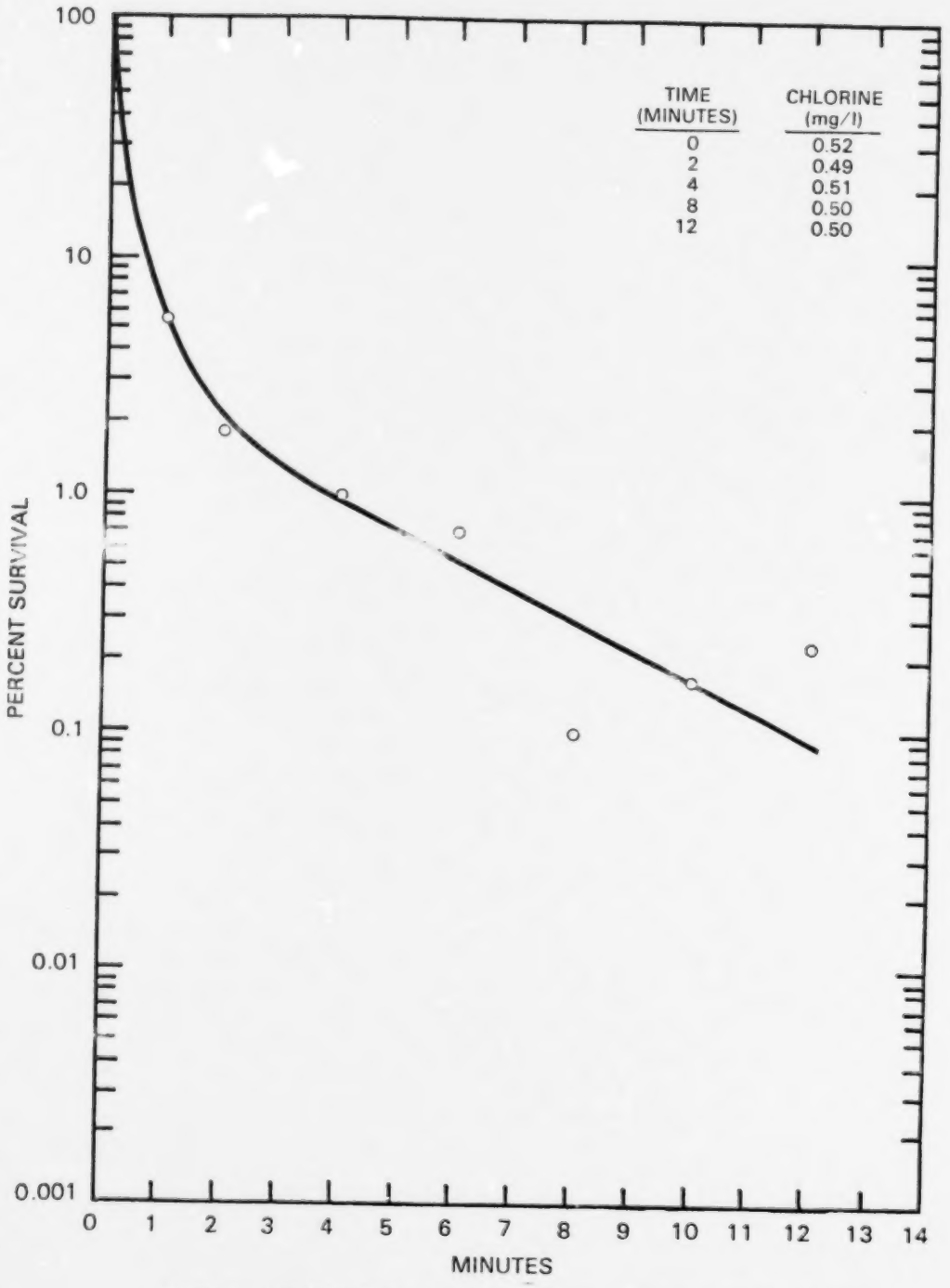

FIGURE 5. THE EFFECT OF 0.5 MG/LITER CHLORINE AT PH 9.9 AND 5°C ON CELL ASSOCIATED SA-11.

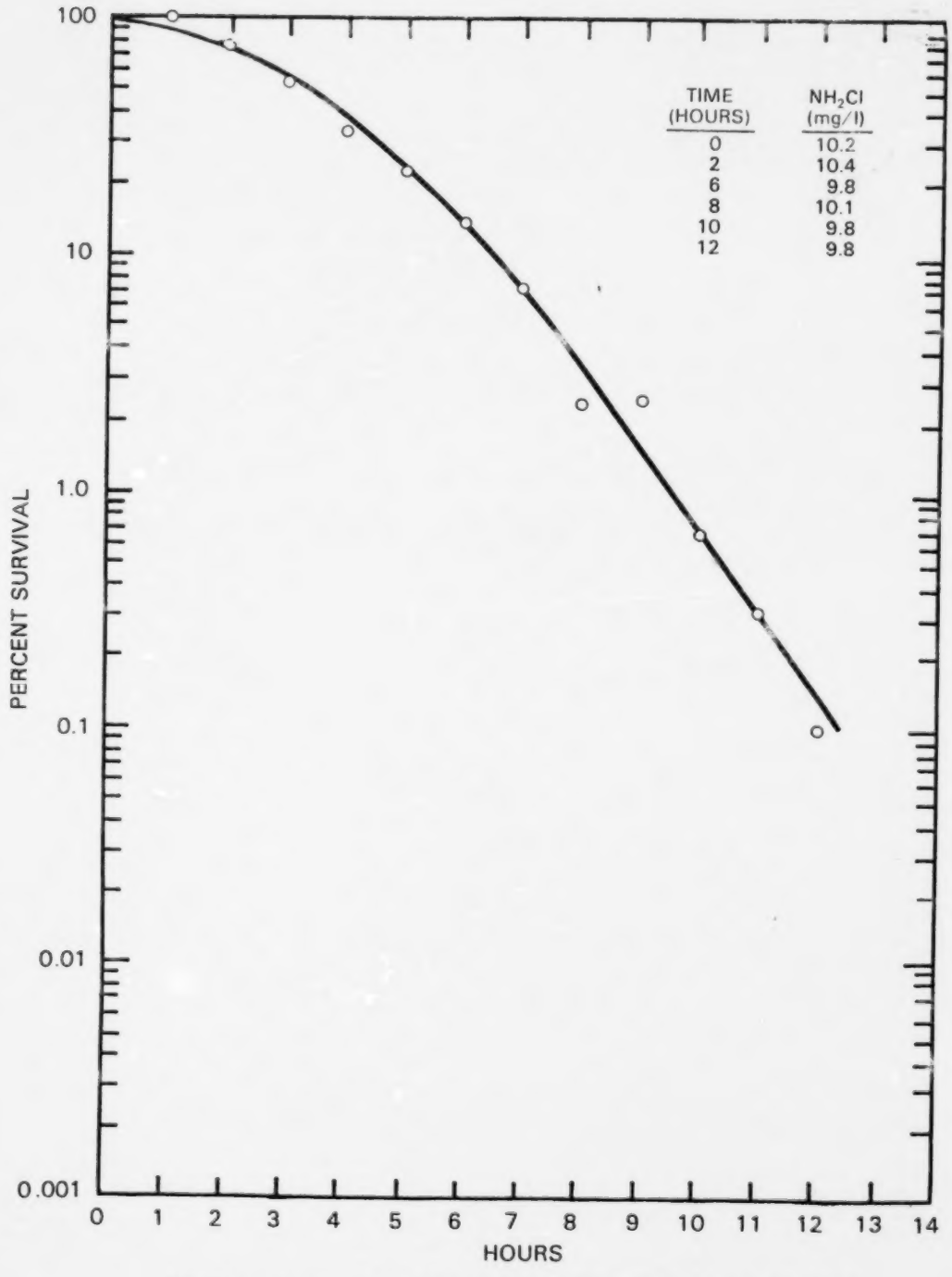

FIGURE 6. THE EFFECT OF 9.8 MG/LITER MONOCHLORAMINE AT pH 8 AND 5°C ON CELL ASSOCIATED SIMIAN ROTAVIRUS SA-11.